ABSTRACT OF THE DISCLOSURE

Methods and compositions for producing single-stranded cDNA (ss-cDNA) in eukaryotic cells, specifically, a DNA cassette that produces ss-cDNA *in vivo*. The cassette contains the Moloney murine leukemia virus reverse transcriptase/RNAse H coding gene, a bacterial restriction endonuclease gene, and a sequence of interest which produces an RNA template from which the reverse transcriptase synthesizes ss-cDNA of a specified sequence. The ss-cDNA is then modified to remove all flanking vector sequences by taking advantage of the "stem-loop" structure of the ss-cDNA, which forms as a result of the inclusion of an inverted tandem repeat that allows the ss-cDNA to fold back on itself, forming a double stranded DNA stem, in the sequence of interest. The double-stranded stem contains one or more restriction endonuclease recognition sites and the loop, which remains as ss-DNA, is comprised of any desired nucleotide sequence. This design allows the double-stranded stem of the stem-loop intermediate to be cleaved by the desired corresponding restriction endonuclease(s) and the loop portion, or sequence of interest, is then released as a linearized, single-stranded piece of DNA. This released (or cleaved) ss-DNA piece does not contain any sequence information either upstream 5' or downstream 3' from the previous double stranded stem portion which contains the restriction endonuclease cut site.

In vivo transfections using the DNA vector constructs described herein demonstrate the use of this system to produce ss-DNA in eukaryotic cells by taking advantage of the many potential promoter(s)/enhancer(s) signals, polyadenylation signals, splice site junctions, ribosome binding sites, and origin of replication signals known to those skilled in the art. The experiments described herein show expression of reverse transcriptase(s)/RNase H(s) and restriction endonuclease(s) within eukaryotic cells, as well as synthesis of RNA transcripts which serve as the template directing the formation of the ss-cDNA for such therapeutic purposes as gene inactivation using duplex or triplex binding of nucleic acids, site-directed mutagenesis, interruption of cellular function by binding to specific cellular proteins, and interfering with RNA splicing functions.